

anion transport by the cholapod family of synthetic anion transporters. [Supported by the EPSRC].

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Designing of Self-Assembled Biomolecular System and the Detection at the Single Molecule Resolution

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Biological system is unique in that component biomolecules are self-organized and emerge adaptable, stable and energy-saving functions. To understand the essential condition for reproducing the properties and constructing stable system from unstable flexible components, we are designing artificial muscle using myosin and DNA nanostructure. System size, spatial positioning, degree of intrinsic noise and the mechanical communication in the synthesized muscle can be modulated and we'll observe the internal dynamics and the system behavior at the single molecule resolution. We have developed several novel tools to modulate the system and monitor the internal dynamics with high precision.

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Experimental Quantification of Fitness Assigned to Cell Lineage Phenotypes

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Recent advance in single-cell measurement techniques has been discovering phenotypic heterogeneity in a clonal cell population quantitatively. To understand biological roles of such phenotypic heterogeneity, it is significant to measure *fitness*, a quantitative representation of cell reproductivity and viability. However, the experimental methods and procedures to determine single-cell-level fitness still remain elusive. Here, we propose a natural definition of fitness whose state variables could be any quantities which can be assigned to single-cell lineages. The fitness function can also be decomposed into division rate, representing reproductivity, and killing rate, representing viability. The advantages of our fitness definition are: (1) the choice of state variables is not limited to the quantities measurable at a single timepoint, but also applicable to the quantities that require the measurement of cellular *dynamics* such as elongation rate and variance of gene expression level; and (2) the fitness can be defined even in the cases where it strongly depends on historical properties of phenotype or the environment is temporally changing.

We first verified the validity of this method by operating simulations of population dynamics with stochastically changing state variables. Next we applied this method to the actual experimental data acquired from timelapse microscopy experiment of synthetic *E. coli* strains expressing fluorescent protein. We thereby investigated the relations among fitness, fluorescent intensity per cell volume and elongation rate. We show that fitness dependence on expression level of antibiotic resistant gene and elongation rate can be successfully quantified using *E. coli* strains that express antibiotic resistant gene as fluorescent-fusion protein.

Such an approach would be useful to clarify connections between population level adaptation and dynamically changing phenotypes at single-cell level.

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Identification of Heavy Metals in Wild Plants Grown on Battery Waste

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The presence of heavy metals in concentrations higher than their natural level in the environment poses a threat to man, his resources and other components that make up the environment. This study was carried out on wild plants and soil in an abandoned slag deposit site of Exide Battery. Lead (Pb), Cadmium (Cd), Nickel (Ni) and Chromium (Cr) in plant shoots, plant roots and soil were determined with "Atomic Absorption Spectroscopy". The trend of accumulation in the plant and soil followed the concentration trend of Pb > Ni > Cr > Cd. The mean concentrations of Pb, Ni, Cr and Cd in the soils were 3262.0 ± 8212.99 mg/kg, 27.89 ± 10.23 mg/kg, 21.65 ± 6.25 mg/kg and 0.60 mg/kg respectively. The mean concentrations of Pb, Ni, Cr, and Cd in plants shoots were 259.21 ± 1012.55 mg/kg, 7.30 ± 10.39 mg/kg, 6.64 ± 10.55 mg/kg and 0.90 ± 1.17 mg/kg respectively. The mean concentrations of Pb, Ni, Cr and Cd in plants roots were: 1515.78 ± 4776.01 mg/kg, 12.86 ± 16.06 mg/kg, 4.60 ± 3.52 mg/kg and 0.76 ± 1.20 mg/kg. From the results obtained it showed that plants have the ability to absorb heavy metals from contaminated soils. Some of these plants had accumulated heavy metals in their tissue but still thrived. The resistant nature and accumulative property of these plants could be looked into and enhanced for phytoremediation.

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Mining Ribozyme-Based Insulators for Incorporation into Genetic not Gates

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A synthetic transcriptional circuit is assembled from individually well-characterized genetic circuit parts. The combined behavior, however, is still hard to predict from the functions of individual parts. One reason is that neighboring genetic sequences can affect part function. Ribozymes are self-cleaving RNAs, and can be used to insulate the interference that occurs at promoter/5'-UTR junctions in post-transcriptional stages. We mined ribozyme-based insulators that consist of two parts: a ribozyme that insulates the promoter/5'-UTR junction and an additional hairpin located immediately downstream from the ribozyme that insulates the 5'-UTR/RBS. Ribozymes are considered to be good insulators if they make circuit element function independent from upstream as well as downstream components. We tested two genes, GFP and CI-GFP, transcribed by two different promoters, pTac and pLacO-1. We show promoter-independence from downstream elements by the linearized relationship between GFP expression and CI-GFP expression under the same promoter. Subsequently, we observe overlapping linearized relationships for two different promoters, indicating that the circuit is decoupled from upstream elements. With this assay, we mined 16 effective ribozyme-based insulators. To incorporate them into genetic circuits, we assign unique combinations of ribozymes, repressors, and terminators to generate unique NOT gates. We then characterized the input-output relationship for these insulated NOT gates.

Synaptic Transmission

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Dynamic Organization of Presynaptic Calcium Channels

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Successful neurotransmitter release depends on the tight association of a release competent transmitter vesicle and the calcium channel. Within small central synapses in the rodent brain the spatial organization of calcium channels has been proposed to be either, a stochastic distribution of single channels or clusters of 10-70 channels within the presynaptic membrane. Both scenarios result in a plausible description of the transmission properties of such synapses, characterized by measurements of the postsynaptic response and/or presynaptic calcium signals. A direct observation of channel activity, number and spatial organization is difficult due to the small size of central synapses.

Here we have created mEOS2-tagged channel constructs, which allow us to investigate the surface population of presynaptic CaV2.1 and CaV2.2-channels within the presynaptic membrane. Overexpression of the tagged alpha1-subunits of calcium channels lead to the clustered localization of the channel subunits in the axonal membrane and colocalised with presynaptic markers as Bassoon or RIM. Overexpression of CaV2.1-channels does not change synaptic channel composition or synaptic activity, but replace up to 90% of the endogenous CaV2.1-channel population, whereas overexpression of CaV2.2-channels does replace the majority of endogenous CaV2.1 and CaV2.2-channels and alters synaptic activity. By using Single-Particle Tracking Photoactivated Localization Microscopy (sptPALM) we could visualize the number and surface dynamics of CaV2.1 and CaV2.2-channels within synapses of cultured hippocampal neurons (14-21 DIV). Within the presynaptic membrane compartment we found about 15-25 tagged channels, ~40% are immobile ($D < 0.001 \mu m^2/s$) and ~60% are mobile for both CaV2.1 and CaV2.2-channels. The immobile fraction is organized within small clusters, whereas the mobile fraction can exchange between clusters and even escape the synapse. This observation supports a stochastic surface distribution of calcium channels within small synapses.

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A Calcium-Independent Oligomerization of Full-Length Synaptotagmin 1 is Mediated by its Juxta-Membrane Linker

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Synaptotagmin 1 (Sytl) is a synaptic vesicle-anchored membrane protein that acts as the major calcium sensor for regulated neuronal exocytosis. A soluble fragment of Sytl (C2AB), which contains the two cytoplasmic C2 domains, has been extensively studied and is generally believed to mimic the main